STUDIES ON MOULDS AND YEASTS IN CREAMERY BUTTER

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PART I

A Study of Media Commonly Employed for the Determination of Moulds and Yeasts in Creamery Butter

Introduction

During the past few years considerable attention has been given to mould and yeast counts in creamery butter. With many laboratories conducting butter analysis on this basis, it is now recognized that some standard procedure should be adopted. While it is admitted that the plate method now in use has its shortcomings, in that it will not yield mathematically correct results, it is, however, necessary to secure an analytical procedure that will furnish comparable figures. Particularly is this necessary where interprovincial mould and yeast competitions are conducted and where the modified butter score card for exhibition butter (1) allows a number of points for mould and yeast counts.

Reviewing the technique as now used among the various workers, it would seem that the most variable factor in the methods for the estimation of moulds and yeasts in creamery butter is undoubtedly the medium. Media as now used may vary in consistency, in sugar and in mineral salt content, and within certain limits, in nutritive value and in hydrogen ion concentration. The fact also that one medium gives a higher count of moulds and yeasts than another is no reason for regarding it as preferable, other factors such as simplicity and uniformity in preparation as well as cost must be considered. It therefore seemed desirable to make a careful study of the media most commonly employed by the various laboratories.

Review of Literature

A review of the literature on mould and yeast counts reveals considerable variation in media employed as to composition, reaction and method of preparation.

Lund (2) employs a medium using as a base unhopped beer wort, acidified by the addition of lactic acid so as to exclude all bacterial growth. Hood & White (3) use a similar medium adjusted to pH 3.5. Hunziker (4), Macy (5), Bouska (6), Parfitt (7), McKay (8), Grimes (9), and Hammer (10) use whey as a nutrient base. These media are all acidified by the addition of either lactic or tartaric acid to no definite pH, with the exception of Parfitt who specifies a pH of 4.6 to 4.8. Stiritz (11) and Abbott (12) claim satisfactory counts using "near beer" as a nutrient base, but to no definite pH. Shutt (13) employs a medium prepared from malt extract base (Panomalt) acidified with lactic acid but to no definite pH.

Apart from composition and hydrogen ion concentration, a wide variation exists as well in methods of preparation of mould and yeast media.

Objects of the Investigation

1. To obtain comparative data on the different media now in use with different hydrogen ion concentration.
2. To obtain comparative data on the different media now in use with a standardized hydrogen ion concentration.

3. To determine the minimum pH that would inhibit all bacterial growth.
4. To work out a "standard" medium or media of uniform composition and reaction, easily prepared and of moderate cost, for mould and yeast counts.

MEDIA SELECTED FOR COMPARATIVE MOULD AND YEAST COUNTS

Experimental Part I

After reviewing the methods used in mould and yeast analysis by other workers, the following media were selected for comparison:—

Medium I. Unhopped wort base. Hood & White.
" II. Bacto wort agar dehydrated.
" III. Whey base. Parfitt.
" IV. Whey base. Bouska.
" V. Whey base. Hammer.
" VI. Malt extract base. (Panomalt). Shutt.
" VII. Bacto whey agar dehydrated.
" VIII. Bacto potato agar dehydrated.

Preparation of Media

The methods given for the preparation of the media are those submitted by the various workers, and were followed as carefully as possible. The dehydrated media were prepared according to the direction of the Digestive Ferments Company, from which all dehydrated media were obtained.

Medium I. Unhopped beer wort is autoclaved and filtered through cotton. 15 grams of agar is dissolved in 600 c.c. of tap water and to this is added 400 c.c. of the beer wort. The medium is put up in 100 c.c. quantities and sterilized. Immediately before plating, 4 c.c. of 5 per cent lactic acid solution is added to each flask. The final pH adjusted to 3.4±.

Medium II. Bacto wort agar dehydrated, original pH 4.7. The pH is reduced to 3.8 by the addition of 2 c.c. of a 5 per cent lactic acid solution to each 100 c.c. of the medium.

Medium III. Whey is obtained from cottage cheese or from milk by curdling with rennet. It is then neutralized to a faint pink using phenolphthalein and brought to a temperature of 210° F. and held for 10 minutes. It is allowed to cool, the albumen settling and the clear whey siphoned off. 1½ per cent of agar and 1 per cent of peptone are added. Autoclave at 15 pounds pressure for 20 to 25 minutes, filter and adjust pH to 6.6 to 6.8. The medium is put in 250 c.c. quantities and sterilized intermittently. The hot agar is acidified using lactic acid 5 per cent to a final pH 4.6 to 4.8

Medium IV. Skim-milk is warmed to about 100° F., acidified with lactic or hydrochloric acid, coagulated with rennet or pepsin, the curd is cut, allowed to settle and then heated to about 115° C. in the autoclave. The whey is filtered off through cotton, neutralized, made up with 1.5 per cent agar, 1 per cent peptone, and filtered. In plating, 1 c.c. of sterile 1 per cent tartartic acid solution (by weight) is placed in the petri dish and 10 c.c. of the medium added. Final pH 4.6.

Medium V. Heat milk to 37° C. Add rennet to coagulate. Break curd and let settle. Drain off whey, strain and measure. Divide whey into equal parts. Add 1.5 per cent agar to 1 part. Add .5 per cent peptone to other

part. Dissolve agar by boiling, then cool. Mix two parts together. Adjust reaction to plus 1. Clear, using eggs. Flask and sterilize. In plating use 1 c.c. of a 1 per cent solution of tartartic acid. Final pH 4.6.

Medium VI. Dissolve 15 grams of agar in 500 c.c. tap water. Dissolve 45 grams of malt extract in 500 c.c. of tap water and steam for 30 minutes. Cool to 60° F. and add egg albumen. Autoclave for 20 minutes at 15 pounds pressure. Filter. Mix the two, flask and sterilize. Acidify at time of plating by adding 4 c.c. of 5 per cent lactic acid to each 100 c.c. of the medium. Final pH 3.6 to 3.8.

Medium VII. Bacto whey agar dehydrated, pH 6.34. The pH is reduced to 3.8 by the addition of 4 c.c. of a 5 per cent lactic acid solution to each 100 c.c. of the medium.

Medium VIII. Bacto potato agar dehydrated, pH 4.6. Medium not acidified.

N.B.—When media are acidified with lactic acid, use U.S.P. lactic acid sp. gr. 1.2, 85 per cent.

Experimental Technique

Samples were taken with sterile triers into sterile four-ounce bottles, two or three plugs being drawn and the top inch of the plug discarded. Samples were held in a Frigidaire at 40° F. until plated. The samples analyzed were representative of fresh and storage butters.

The butter samples were brought to a creamy consistency in a water bath at 110°-115° F. and the water blanks were heated to a similar temperature. Plates were made of 1 c.c. and a dilution of 1/100 c.c. of each sample for all media.

The media were acidified in the flasks immediately before plating with the amount and kind of acid stipulated in methods of preparation. The pH of the media was determined by the colorimetric method with a LaMotte hydrogen ion testing set, using bromphenol blue as an indicator.

The plates were inverted and incubated at a temperature of 25° C. for five days.

Counting was done with the naked eye and all colonies showing on the plates were included. Results were recorded as the number of moulds and yeasts per c.c. of butter.

When the plates of each medium had been counted, twelve colonies were picked from representative plates, stained and examined microscopically. This procedure was adopted to determine if the pH of the media was sufficiently low to inhibit bacterial growth. The examination of colonies in this way did not give a proportionate count of yeasts and bacteria, but it sufficed to show roughly the extent of bacterial growth on each medium.

TABLE I.—COMPARISON OF MOULD AND YEAST COUNTS ON SELECTED MEDIA—EXPERIMENTAL PART I.

Sample Number	Medium I pH 3·5		Medium II pH 3·7		Medium VI pH 3·7		Medium VII pH 3·8		Medium III pH 4·6		Medium IV pH 4·6		Medium V pH 4·6		Medium VIII pH 4·6	
	*Moulds	*Yeasts	Moulds	Yeasts	Moulds	Yeasts	Moulds	Yeasts	Moulds	Yeasts	Moulds	Yeasts	Moulds	Yeasts	Moulds	Yeasts
1.....	5·0	107·0	3·5	123·0	2·0	136·0	2·5	129·0	2·5	64,800	3·0	96,500	5·5	82,800	1·5	65,200
2.....	0·5	65·0	2·0	90·0	2·0	69·0	1·5	79·0	3·0	29,700	0·5	124,000	1·5	76,800	2·0	160,000
3.....	70·0	203·0	82·0	209·0	70·0	194·0	66·0	1,000·0	52·0	20,400	59·0	84,000	71·0	108,400	73·0	97,200
4.....	25·0	31·5	24·0	28·0	36·0	42·0	20·0	232·0	19·0	11,900	29·0	30,400	33·0	78,200	19·0	100,000
5.....	9·5	115·0	4·5	176·0	3·5	118·0	8·0	150·0	6·0	7,400	8·5	6,000	7·0	.....	6·0	.....
6.....	42·5	108·0	38·5	132·0	45·0	111·0	35·0	700·0	28·0	10,000	27·5	12,000	29·0	10,000	42·5	10,500
7.....	12·5	54·0	7·0	76·0	9·0	59·0	6·5	93·0	12·0	4,200	6·0	3,900	10·0	7,800	8·0	5,500
8.....	11·5	89·5	10·5	111·0	16·0	62·0	15·5	79·5	16·0	32,400	16·5	45,500	15·0	50,000	16·0	84,400
9.....	4·0	11·5	2·0	22·5	3·0	18·0	2·5	49·0	3·5	1,400	4·5	5,550	3·0	4,000	2·5	4,750
10.....	22·0	84·0	18·5	86·0	16·0	80·5	16·0	85·0	18·5	16,600	21·0	19,000	23·5	18,400	16·5	41,000
11.....	24·0	198·5	14·5	216·0	19·5	208·0	29·5	168·0	25·5	9,600	20·0	40,600	24·5	38,700	18·5	48,800
12.....	4·0	31·5	3·5	50·0	3·5	39·5	4·0	54·0	5·0	12,800	5·0	16,200	4·5	20,400	4·5	36,000
13.....	0·0	12·5	0·5	43·5	0·5	24·0	0·0	50·5	1·0	1,450	1·5	4,100	1·5	6,200	0·5	8,800
14.....	2·0	57·0	2·5	61·0	0·5	71·0	2·0	52·5	3·5	22,100	2·0	22,800	0·5	43,100	1·0	34,000
15.....	2·5	68·5	3·5	109·5	2·5	95·5	9·0	89·5	9·5	39,000	9·5	43,400	7·0	63,600	2·5	61,200
16.....	1·5	38·5	2·5	44·0	3·0	41·0	5·5	101·0	3·5	60,800	2·5	69·000	1·5	73,200	3·0	70,400
17.....	0·5	15·0	1·5	8·0	1·0	16·0	6·0	14·5	1·5	1,500	1·0	2,100	1·0	2,050	1·0	2,800
18.....	3·5	1,950·0	3·0	2,250·0	4·0	2,300·0	9·0	3,000·0	4·5	29,600	4·5	222,000	7·0	210,000	7·0	153,400
19.....	0·5	12·0	1·0	13·0	0·5	12·5	1·0	10·0	3·0	350	2·5	1,200	3·0	396	1·5	1,250
20.....	3·0	35·0	2·0	62·0	2·0	49·5	0·5	90·0	4·5	20,600	1·0	21,800	2·0	15,500	2·5	15,150
21.....	0·5	19·0	1·0	25·5	0·0	26·0	2·0	41·5	0·5	5,050	0·5	8,450	0·0	7,450	1·0	9,600
22.....	300·0	2,100·0	500·0	2,200·0	250·0	2,500·0	300·0	9,250·0	450·0	108,000	350·0	112,000	300·0	119·000	250·0	136,000
23.....	850·0	5,800·0	850·0	5,050·0	550·0	5,750·0	1,050·0	8,500·0	1,050·0	113,600	800·0	125,200	950·0	126,400	900·0	137,600
24.....	74·0	2,400·0	51·0	2,700·0	67·0	2,100·0	87·0	2,600·0	61·0	100,000	.....	100,000	200·0	100,000	80·0	120,000
25.....	42·0	7,100·0	40·0	8,300·0	51·0	8,500·0	53·0	8,300·0	41·0	100,000	3·5	100,000	.....	100,000	63·0	100,000

*Moulds and yeasts per c.c. of butter.

Discussion of Results of Experimental Part I

In table I are given the mould and yeast counts of the twenty-five individual samples on the media used in experimental part I. There is considerable variation in the counts of the same sample on the various media used. This variation is very noticeable when the counts on the media with a pH of 4.6 are compared with the counts on the media with a pH of 3.5 to 3.8, and especially so in the case of the counts given as yeasts.

This is clearly shown in table II, which gives the average mould and yeast counts for the twenty-five samples on each medium.

With the exception of medium VI the average mould counts are fairly uniform, but the average counts given as yeasts showed a very great increase on the media with a pH of 4.6 when compared with the average counts on the media with a pH of 3.5 to 3.8.

It will also be noticed that the average yeast count on medium III was much lower than for other media of similar pH. This was probably due to the fact that it was found difficult to obtain a clear medium even when following directions of preparation carefully, and it is possible that many small subsurface colonies were missed in counting.

In counting the plates where media with a pH of 4.6 were used, many small colonies were observed. On examination many of these colonies were found to be bacteria and not yeasts. These bacterial colonies were included in the yeast counts, and accounted for the high counts on the media having a pH of 4.6.

When the colonies from the media with a pH of 3.4 to 3.8 were examined, very few, if any, bacterial colonies were present. On medium I plates from only two samples showed bacterial colonies; on medium II plates of 4 samples showed bacteria present; on medium VI none of the colonies examined were bacteria; and on medium VII a few bacterial colonies were found on plates of 6 samples. On the different whey media and potato agar which had a pH of 4.6, there was considerable bacterial growth. In the majority of cases, out of twelve colonies that were examined from these media, nearly all were bacteria, indicating a relatively large number of bacterial colonies that were included in the yeast counts.

This is more clearly illustrated in table III.

TABLE II.—AVERAGE MOULD AND YEAST COUNTS ON SELECTED MEDIA.—EXPERIMENTAL PART I

No.	Medium	Moulds per c.c.	Yeasts per c.c.	pH of Media
I	Beer wort agar, Hood & White	60·4	828	3·5±
II	Bacto dehydrated wort agar	66·8	887	3·7±
VI	Malt agar. (Panomalt). Shutt	46·3	905	3·7±
VII	Bacto dehydrated whey agar	69·3	1,397	3·8±
III	Whey agar. Parfitt	72·9	32,930	4·6±
IV	Whey agar. Bouska	58·8	52,628	4·6±
V	Whey agar, Hammer	70·9	56,766	4·6±
VIII	Bacto dehydrated potato agar	60·9	63,064	4·6±

TABLE III.—RELATION OF pH OF MEDIA TO BACTERIAL COLONIES—EXPERIMENTAL PART I

Sample	Medium I pH 3·5±	Medium II pH 3·7±	Medium VI pH 3·7±	Medium VII pH 3·8±	Medium III pH 4·6±	Medium IV pH 4·6±	Medium V pH 4·6±	Medium VIII pH 4·6±
1	0	1	0	0	11	11	12	9
2	0	2	0	0	10	9	11	12
3	0	0	0	0	10	7	10	11
4	0	0	0	7	10	11	12	10
5	0	0	0	0	8	10	12	8
6	0	0	0	0	6	7	8	8
7	0	0	0	0	12	10	12	10
8	0	0	0	0	12	11	10	11
9	0	0	0	0	9	12	8	5
10	0	0	0	0	12	9	10	11
11	0	0	0	0	11	12	10	11
12	0	0	0	1	11	12	12	12
13	0	0	0	0	12	11	11	12
14	0	0	0	0	12	11	11	12
15	0	0	0	0	10	10	10	12
16	3	4	0	4	12	10	12	11
17	1	5	0	0	12	12	12	12
18	0	0	0	0	11	4	3	7
19	0	0	0	0	7	9	6	6
20	0	0	0	0	12	11	11	12
21	0	0	0	0	12	12	12	12
22	0	0	0	2	10	9	11	9
23	0	0	0	1	10	9	6	6
24	0	0	0	1	2	10	9	3
25	0	0	0	0	1	12	6	10

Experimental Part II

As there was considerable variation in the mould and yeast counts on the media when the pH differed, it was thought advisable to make further analyses using the same media and standardizing the pH. For experimental part II six of the media used in experimental part I were selected with the addition of a new dehydrated malt extract agar manufactured by the Digestive Ferments Company. In this part of the work, all the media were standardized to a pH of 3.5± with a 5 per cent solution of lactic acid (sp. gr. 1.2, 85%).

A standard pH of 3.5± was selected because it had been the experience of the authors that such a pH permitted good growth of yeasts and moulds and at the same time inhibited bacterial growth. The growth of some types of butter yeasts may be inhibited also at a pH of 3.5± which would introduce some inaccuracy in the yeast counts. However, any inaccuracy from this source is undoubtedly much less than would occur if the pH of the media permitted considerable bacterial growth to take place.

This question of whether a pH of 3.5± for yeast and mould media inhibits the growth of some types of yeasts common to butter is being investigated further by the Division of Dairy Research.

Below is given the media used in experimental part II with the amount of lactic acid solution used per 100 c.c. of medium to obtain a final pH of 3.5±.

MOULD AND YEAST MEDIA—EXPERIMENTAL PART II

No.	Medium	Number c.c. of 5 p.c. lactic acid sol. per 100 c.c. media	Final pH
I	Beer wort agar. Hood & White	4·0 c.c.	3·5±
II	Bacto dehydrated wort agar	2·5 c.c.	3·5±
III	Bacto dehydrated malt extract agar	2·5 c.c.	3·5±
IV	Malt agar. (Shutt)	4·5 c.c.	3·5±
V	Bacto dehydrated whey agar	5·5 c.c.	3·5±
VI	Whey agar	10·0 c.c.	3·5±
VII	Bacto dehydrated potato agar	4·0 c.c.	3·5±

In table IV are given the mould and yeast counts of each sample on the different media used in experimental part II. In table V are given the average counts for each medium used.

The average mould and yeast counts check closely and are comparable when allowance is made for the probable error in the plate method.

Colonies were examined from plates of each medium as in experimental part I but no bacterial colonies were observed on examination.

TABLE IV.—COMPARISON OF MOULD AND YEAST COUNTS ON SELECTED MEDIA.—EXPERIMENTAL PART II

Sample Number	Medium I pH 3·5 ±		Medium II pH 3·5 ±		Medium III pH 3·5 ±		Medium IV pH 3·5 ±		Medium V pH 3·5 ±		Medium VI pH 3·5 ±		Medium VII pH 3·5 ±	
	*Moulds	*Yeasts	Moulds	Yeasts	Moulds	Yeasts	Moulds	Yeasts	Moulds	Yeasts	Moulds	Yeasts	Moulds	Yeasts
1	6	132	6	205	6	136	5	110	7	117	7	126	10	134
2	34	3,300	32	3,600	32	3,800	24	2,800	36	2,400	28	1,200	34	2,800
3	1	81	2	116	2	109	2	81	2	89	2	71	1	97
4	1	81	0	74	1	83	3	73	2	117	3	99	2	98
5	17	107	11	160	11	106	15	124	15	107	10	98	25	133
6	12	112	12	115	12	107	14	112	13	130	9	115	10	94
7	8	**	13	**	3	**	23	**	6	**	12	**	11	**
8	5	**	3	**	2	**	2	**	7	**	4	**	3	**
9	37	121	28	114	36	120	17	115	38	105	26	105	42	103
10	5	13	16	59	11	24	13	26	6	23	4	20	15	33
11	15	38	12	31	10	33	14	32	14	3	0	42	14	21
12	2	18	3	21	1	19	2	20	0	22	0	21	4	18
13	41	214	24	113	5	34	4	22	14	67	0	21	29	182
14	28	31,200	22	31,400	20	29,300	22	25,600	19	29,600	18	25,900	29	29,500
15	2	11,500	2	10,300	0	11,500	6	7,600	3	9,600	3	8,900	1	10,600
16	18	48,000	22	48,600	9	55,700	11	48,400	18	56,000	15	49,600	18	45,600
17	22	48,400	27	55,200	18	42,400	13	61,200	25	55,100	16	55,000	10	51,900
18	11	154	8	153	10	103	10	111	11	93	8	101	6	155
19	12	229	8	219	10	256	8	200	9	159	6	161	10	194
20	1	6,100	1	7,500	0	6,400	0	5,600	0	6,800	0	5,200	0	9,700
21	0	1,500	0	2,300	0	2,300	0	1,600	0	2,900	0	1,900	0	2,500
22	2	169	4	197	4	198	3	194	3	198	4	100	2	312

*Moulds and yeasts per cc. of butter.

**Over 100,000 yeasts per cc.

TABLE V.—AVERAGE MOULD AND YEAST COUNTS ON SELECTED MEDIA—EXPERIMENTAL PART II

Medium	Moulds per c.c.	Yeasts per c.c.
Wort agar. Hood & White	12·7	15,976
Dehydrated wort agar	11·6	16,385
Dehydrated malt extract agar	9·2	16,033
Malt agar. Shutt	9·6	16,092
Dehydrated whey agar	11·3	16,530
Whey agar	8·0	15,854
Dehydrated potato agar	12·5	16,099

Summary

1. In experimental part I the most variable factor affecting counts was found to be the pH when all media were prepared and acidified according to the methods outlined by the various workers.
2. In counting, large numbers of bacterial colonies were present on media of pH 4.6 and a few colonies on media of pH 3.8.
3. When counts on media of pH 3.8 were compared with counts on media of pH 4.6, wide differences in the average yeast counts were found, due to counting bacterial colonies as yeasts on media of pH 4.6.
4. In experimental part II the mould and yeast counts on individual samples and the average counts compared favourably with one another when all media were of the same pH value, 3.5.
5. All bacterial growth was inhibited at pH 3.5.
6. When wort, malt, whey and potato were used as a nutrient base with a pH of 3.5, they were found to be equally suitable for the growth of moulds and yeasts.
7. Dehydrated wort, malt extract, and home made beer wort media were more easily prepared and gave clearer media than the home made whey agar.
8. No data are available on the comparative cost of dehydrated and home made media.

PART II

A Tentative Standard Method for the Determination of Moulds and Yeasts in Creamery Butter

Introduction

The rapid development in the use of laboratory methods for controlling the mould and yeast counts of pasteurized butter makes necessary the adoption of a standard procedure for routine analysis.

While it is recognized that the American Dairy Science Association, through its committee (14) on bacteriological methods, is contemplating a complete set of microbiological procedures useful in controlling the quality of all dairy products, these methods are not yet available. To secure uniformity of technique for the coming year on interprovincial mould and yeast competitions and mould and yeast counts in exhibition butter, the following procedure will be adopted tentatively.

Procedure

Sampling.

(1) Fourteen and fifty-six-pound packages.

About one ounce of butter is removed from two different corners of the package with a sterile metal spatula or spoon, in such manner as to include portions of the top and two sides of the package. This is transferred to a sterile four-ounce screw top sample jar. A separate sterile spatula or spoon is used for each lot of butter sampled.

(2) Print butter.

Cut the print in halves with a sterile knife, cut a slab one quarter of an inch thick from the cut surface, and transfer to a sterile four-ounce screw top sample jar.

Care of Samples.

All samples should be placed in cracked ice immediately after taking or placed in a cold room where the temperature does not exceed 38° F., and plated as soon as possible.

Where samples are secured at grading station remote from the laboratory, they should be stored at 15° F. or lower until shipped. No samples should be held longer than one week before shipping. Shipping should be made in an iceless ice cream shipping container and plated as soon as possible on receiving.

Preparation of Sample.

Place the sample bottle containing the butter to be tested in a water bath between 40° and 45° C. and shake until a creamy consistency of the butter is secured. The length of time the sample is in this creamy consistency must not exceed fifteen minutes.

Medium.

Bacto malt agar dehydrated prepared by the Digestive Ferments Company of Detroit shall be used and prepared according to the directions on the container.

The medium should be dispensed in 100 c.c. quantities in 150 c.c. flasks and sterilized by autoclaving at 15 pounds pressure for 20 minutes. Before pouring, the medium is acidified according to directions on the container and checked by one of the recognized colorimetric methods with the use of bromphenol blue and again adjusted if necessary to a final pH of 3.5±.

Plating.

With a previously warmed 1 c.c. pipette the melted butter is drawn up into the pipette and delivered into the petri dish, blowing hard to remove as much of the contents as possible. A 1/10 dilution shall be made by the addition of 1 c.c. of butter to a cotton stoppered test tube containing 9 c.c. of sterile water at a temperature of 40° to 45° C. and mixed by drawing the sterile water into the pipette four or five times, keeping the tip of the pipette submerged.

Ten c.c. of the melted agar at a temperature of 40° to 45° C. should be poured into each petri dish and thoroughly mixed with a rotary motion.

The plating should be completed as rapidly as possible.

After the agar has been thoroughly hardened, place inverted petri dish in an incubator.

Incubation.

Plates are inverted and incubated at 25° C. for five days.

Method of Counting.

Count with the naked eye the number of moulds and the number of yeasts per plate. If moulds develop in large numbers, counts should be made after two or three days and a recount on the fourth or fifth day.

Reporting Results.

Results to be expressed as the number of yeasts and moulds per gram or cubic centimeter of butter separately and together as a total count.

PART III

Application of the Modified Score Card for Exhibition Butter

Introduction

Buttermakers and judges of butter are all agreed that butter for exhibition should represent the very highest type that can be produced. Such being the case, butter then must be high scoring in flavour and workmanship. With the inception and application of the mould and yeast count in Ontario and the western provinces, to the manufacture of pasteurized butter an added feature has been introduced which extends workmanship beyond texture, incorporation of moisture, colour, salt and package, to one of sanitary conditions of the plant and equipment. From mould and yeast counts received from the various provincial laboratories during the past summer, commercially pasteurized butters are now beginning to conform more closely to the standards set, namely, 0-10, excellent; 10-50, good; 50-100, fair; and over 100, poor. Unfortunately, most exhibition butters do not begin to meet this standard. The answer to this is simple, the present official score gives no credit for this important phase of workmanship from the standpoint of sanitation.

If exhibition butter then is to represent the very highest type of butter that can be manufactured, this condition should not exist, and as an incentive for greater care and vigilance in cleaning equipment, and to encourage the most efficient sanitary methods of handling the product, and in order that credit be given to the painstaking worker, a modification of the present score card is suggested for all exhibition butter in Canada.

The concensus of opinion among butter judges is that perfection has now been reached in exhibition butter on points for salt and package. Such being the case, and in view of the significance of the mould and yeast count in exhibition butter, a reduction of 5 points on the items of salt and package seems desirable, and that the mould and yeast count be introduced into the modified score card with a total of 10 points.

Modified Score Card

The following is the score card proposed for exhibition butter:—

	points
Flavour	45
Texture	15
Incorporation of moisture	10
Colour	10
Salt	5
Package	5
Yeast and mould count	10
Total	100

The application of the mould and yeast count to the modified score card is as follows:—

Moulds and Yeasts	Points	Moulds and Yeasts	Points
10 and less	10·0	400 and less	7·5
20 and less	9·9	500 and less	7·0
30 and less	9·8	600 and less	6·5
40 and less	9·7	700 and less	6·0
50 and less	9·6	800 and less	5·5
60 and less	9·5	900 and less	5·0
70 and less	9·4	1,000 and less	4·5
80 and less	9·3	2,000 and less	3·5
90 and less	9·2	3,000 and less	2·5
100 and less	9·1	4,000 and less	1·5
150 and less	8·8	5,000 and less	0·5
200 and less	8·5	Over 5,000	0·0
300 and less	8·0		

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